

Characterizing the Achilles tendon mechanoreponse to voluntary wheel running

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Introduction: Tendons act as a mechanosensitive bridge that transmit contractile muscle forces to bone to enable movement and maintenance of body posture. Tendons have historically been regarded as inert tissues, but recent work suggests that tendons respond and adapt to physiological mechanical load by promoting collagen synthesis and improving functional biomechanical properties [1]. While mechanical force is critical to maintaining tendon homeostasis, chronic or repetitive loading puts tendons at risk for tendinopathy and over-use injuries. Previous studies implicate tenocytes as the main facilitators of mechanotransduction, however the cellular mechanisms underpinning this process and how the subsequent tissue-level adaptive or maladaptive mechanoreponse is mediated remain unknown [2]. The goal of the present study was to evaluate voluntary wheel running (VWR) as a model of physiological mechanical load that could be used to identify possible candidates regulating both tendon homeostasis and damage in response to mechanical load.

Methods: The University Committee on Animal Resources approved all animal studies. *Mice:* 10-week-old male C57BL/6J mice were obtained from Jackson Laboratory. *VWR set up:* Individually housed mice were allowed to freely run on the open surface of a slanted plastic saucer shaped wheel inside the mouse cage [3] for 8 or 12 weeks. Wheel rotations were continuously recorded electronically to record the running frequency and rate. Sedentary control mice were placed in cages with a locked wheel. *Bulk RNA-seq:* After 8 and 12 weeks (n=7 per timepoint and exercise regimen), Achilles tendons were isolated, and flash frozen in liquid nitrogen. Individual tendons were homogenized (PowerMasher II) and total RNA was isolated (Direct-zol RNA Microprep kit) for bulk-RNA sequencing. *Transcriptomic analysis:* Data normalization and differential expression analysis of VWR mice relative to locked controls at a given timepoint was performed using DeSeq2.1 with an adjusted p-value < 0.05 on each set of normalized expression measures. Significantly upregulated and downregulated differentially expressed genes (DEGs) were entered into the Database for Annotation, Visualization, and Integrated Discovery (DAVID) for gene ontology (GO) analysis.

Results: *VWR kinetics:* After an acclimatization period (weeks 1-4), running behavior stabilized at 5 weeks (123.8 ± 13.5 km/week at 5 weeks) before reaching an average of 91.59 ± 5.1 km/week for weeks 8-12 (Fig. 1). Over the course of the experiment, the 8-week VWR cohort averaged a total run distance of 783.09 ± 49.0 km and the 12-week VWR cohort averaged a total run distance of 1111.0 ± 131.1 km.

VWR mice displayed distinct transcriptional shifts at both 8 and 12 weeks: After 8 weeks of running, 24 genes were upregulated, and 22 genes were downregulated in the Achilles tendons of VWR mice relative to sedentary controls (Fig. 2A,B). After 12 weeks of running, 97 genes were upregulated and 139 genes were downregulated, relative to sedentary controls (Fig. 2D,E). *After 8 weeks of running, Achilles tendons exhibit an anabolic adaptive response:* GO analysis of DEGs revealed that after 8 weeks of running, VWR Achilles tendons upregulate pathways relating to morphogenesis, embryogenesis and cell adhesion processes (Fig. 2C), suggesting a developmentally directed adaptation process. No significantly enriched downregulated pathways were observed. *By 12 weeks, Achilles tendons shift their transcriptional response to loading:* In contrast to the 8 week VWR cohort, processes involving morphogenesis, development and matrix remodeling were downregulated in 12 week VWR mice relative to sedentary controls (Fig. 2G). Upregulated GO terms include heat generation and metabolic processes (Fig. 2F). Together, these results suggest that after 12 weeks of running, VWR Achilles tendons upregulate metabolism related mechanisms to regulate available energy and potentially adjusts towards a homeostatic adaptive response.

Discussion: Numerous studies have implicated tenocytes as the mechanosensitive intermediary in tendon, but little is known about exactly how tendons translate mechanical signals into a tissue-level adaptive response. The results of our study suggest that in response to mechanical loading, the Achilles tendon promotes adaptation via developmentally guided processes after 8 weeks but downregulates similar mechanisms after 12 weeks of physiological load. This may suggest that 8 weeks of mechanical loading is sufficient to promote morphogenesis and drive anabolic adaptation, but after 12 weeks of loading, these processes potentially shift toward a new homeostatic state, indicating homeostatic adaptation by downregulating developmental processes. Taken together, this suggests that a switch between adaptive anabolic growth and adaptive homeostasis may occur between 8 and 12 weeks of physiological loading. Ongoing functional, structural, and morphological studies will shed light on how these transcriptional responses to VWR affect overall tendon behavior. Importantly, the VWR setup takes advantage of the natural inclination of mice to run without any additional stress, therefore the observations from this study represent a physiological response. The goal of future studies will be to use this model to parse out the complex mechanotransductive network that allows tenocytes to coordinate a tissue-level adaptive response.

Significance: Establishing a model of physiological mechanical load in Achilles tendon will allow for future characterization of the tenocyte mechanotransduction network.

References: 1. Bohm et al. *Sports Medicine*, 2015. 2. Goh et al. *Curr Protoc Mouse Biol*, 2015. 3. Manzanares et al. *Braz J Med Biol Res*, 2018. 4. Hostrup et al. *Comprehensive Physiology*, 2021.

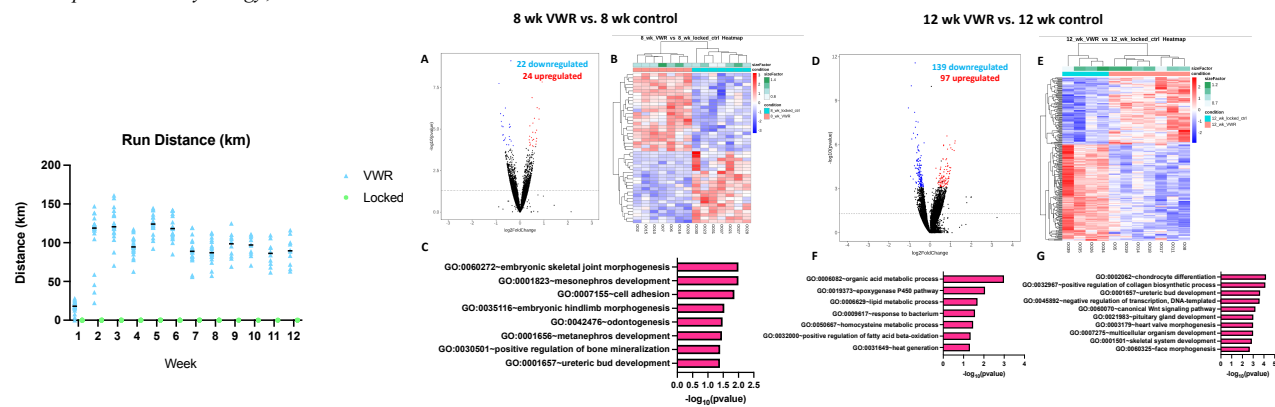


Fig. 1: Mice reach a plateau in running distance (km) after 5 weeks.

Fig. 2: Representation of DEGs between 8-week and 12-week VWR mice relative to sedentary controls, respectively. Volcano plots (A,D) depict significantly upregulated DEGs as red dots and significantly downregulated DEGs as blue dots. Heat maps (B,E) represent all significant DEGs, with the data representing the log transformation of the normalized count data. Identification of GO terms that are significantly enriched in upregulated (C,F) and downregulated (G,H) DEG.